

On the importance of accounting for intraspecific genomic relatedness in multi-species studies

Simon Joly^{1,2}, Dan F. B. Flynn^{3,4}, and Elizabeth Wolkovich^{3,4}

¹*Montreal Botanical Garden, Montréal, Canada*

²*Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, Montréal, Canada*

³*Department of Organismic & Evolutionary Biology, Harvard University, MA, USA*

⁴*Arnold Arboretum, Harvard University, MA, USA*

Running Head: Intraspecific genetic correlations in mixed models

1 Abstract

- 2 1. Analyses in many fields of ecology are increasingly considering multiple species and multiple
3 individuals per species. Premises of statistical tests are often violated with such datasets be-
4 cause of the non-independence of residuals due to phylogenetic relationships or intraspecific
5 population structure. If comparative approaches that account for the phylogenetic relation-
6 ships of species are well developed and their benefits demonstrated, the importance of con-
7 sidering the intraspecific genetic structure, especially in combination with the phylogenetic
8 structure, has rarely been addressed.
- 9 2. We investigated whether it is beneficial to account for intraspecific genomic relatedness in
10 multi-species studies. For this, we used a Phylogenetic Mixed Model to analyze first a suite of
11 simulated data and then results from one example ecological study—a budburst experiment
12 where clippings of 10 tree and shrub species were subjected to different temperatures and
13 photoperiods.
- 14 3. We found that accounting for intraspecific genetic structure yields more accurate and precise
15 fixed effects as well as increased statistical power, but more so when the relative importance
16 of the intraspecific to the phylogenetic genetic structure is greater. Analysis of the budburst
17 experiment further showed that accounting for intraspecific and phylogenetic structures yields
18 improved estimates of warming and photoperiod effects and their interaction in explaining
19 the time to budburst.
- 20 4. Our results show that statistical gains can be made by incorporating information on the
21 intraspecific genomic relatedness of individuals in multi-species studies. This is relevant for
22 investigations that are interested in intraspecific variation and that plan to include such
23 observations in statistical tests.

24 **Key-words:** Phylogenetic mixed models (PMM), genetic structure, intraspecific variation, leaf
25 phenology, climate change, phylogenetic generalized least squares (PGLS).

26 Introduction

27 The reactions of different species to external stimuli are not independent. Because physiological
28 responses have a genetic basis, closely related species are more likely to have similar responses to a
29 specific treatment. This phylogenetic non-independence of species responses violates the assump-
30 tions of most statistical tests, such as the independence of residuals in regression, and negatively
31 impacts the results in terms of parameter estimates and p-values (e.g., Revell, 2010). This has been
32 recognized for some time and a family of methods—comparative methods—have been developed to
33 address this problem (Felsenstein, 1985; Grafen, 1989; Lynch, 1991; Ives et al., 2007; Felsenstein,
34 2008; Revell, 2010; Hadfield and Nakagawa, 2010).

35 Recently there has been growing awareness among ecologists of the need to also consider in-
36 traspecific variation within ecophylogenetic analyses. Within community ecology, there have been
37 calls to increase studies of intraspecific trait variation (Violle et al., 2012; Alofs, 2016) and to
38 develop the necessary statistical models for such multilevel data (Funk et al., 2017; Read et al.,
39 2016). Similarly, studies of climate change have repeatedly highlighted the need for models that
40 incorporate variation in responses across both species and populations (Willis et al., 2008; Char-
41 mantier et al., 2008; Anderson et al., 2009; Chen et al., 2011). However, little attention has been
42 given to the genetic correlation structure present below the species level within the field of com-
43 parative methods (but see Hansen et al., 2000; Felsenstein, 2002; Stone et al., 2011; Read et al.,
44 2016; Garamszegi, 2014). Moreover, studies rarely account for both phylogenetic and intraspecific
45 genetic correlations simultaneously, even though the sampling structure in many ecological studies
46 calls for such a design.

47 Presently, studies that account for phylogenetic correlation almost always ignore intraspecific
48 genetic structure and as such assume that intraspecific samples are drawn from a single population.
49 In contrast, many ecological studies explicitly sample individuals across important geographical
50 ranges or from populations among which gene flow could be restricted, resulting in a potentially
51 non-trivial correlation structure among samples. If this correlation is important, statistical tests

52 that do not account for it are expected to be biased.

53 Until recently, the difficulty of obtaining genetic data to accurately estimate intraspecific genetic
54 correlations provided sufficient justification for ignoring this source of variance in ecological stud-
55 ies. But the rapid development of sequencing techniques now allows precise estimation of genetic
56 relatedness (Gienapp et al., 2017), at relatively affordable prices. This could allow a better under-
57 standing of how ecological responses are influenced by the genetic relationships among species and
58 populations at once. One area where this potential is particularly high is climate change research,
59 where evidence of rapid ecological and evolutionary change is growing. Research has highlighted
60 that species responses to climate change appear phylogenetically patterned, with species from cer-
61 tain clades and with particular traits appearing most vulnerable to local extinctions with warming
62 (Willis et al., 2008). At the same time other work has highlighted discrepancies in species re-
63 sponses when studied over space (Charmantier et al., 2008), suggesting populations within species
64 may show different responses to climate change. This is supported by population-level research
65 that has found large differences in the range shifts of northern versus southern populations with
66 warming (Anderson et al., 2009; Chen et al., 2011). Such results make clear that the best estimates
67 of responses will require methods that consider variation at both the species and population levels,
68 and the connections between different populations and different species, all at once.

69 The objectives of this report are to assess the importance of accounting for intraspecific genetic
70 correlations in ecological studies. To achieve this, we use the phylogenetic mixed model (PMM)
71 that allows multiple levels of genetic structure to be considered simultaneously (Lynch, 1991; Hous-
72 worth et al., 2004; Hadfield and Nakagawa, 2010). Other approaches can be used to account for
73 intraspecific correlations (reviewed in Stone et al., 2011; Garamszegi, 2014), but as we show below
74 none currently provides as much flexibility as the PMM. We assess the importance of accounting
75 for intraspecific genetic correlation structure using simulated data and provide a climate change-
76 related empirical example where we investigate the importance of temperature and photoperiod on
77 the timing of budburst of ten tree and shrub species.

78 **Methods**

79 **The phylogenetic mixed model**

80 The phylogenetic mixed model has been described in detail elsewhere (Housworth et al., 2004; Had-
81 field and Nakagawa, 2010; Villemereuil and Nakagawa, 2014), thus our description here is brief and
82 focuses on the inclusion of phylogenetic and intraspecific correlations structures as random effects
83 in the model and on the inclusion of fixed effects. In the following, we assume that phylogenetic and
84 intraspecific correlations have been estimated independently, which allows the two structures to be
85 included as separate effects and to quantify their relative importance. Here lowercase italic letters
86 represent numbers, lowercase boldface letters vectors and uppercase boldface letters matrices. The
87 phylogenetic mixed model (PMM) has the form:

$$\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{b} + \mathbf{e}, \quad (1)$$

88 where \mathbf{y} is the response variable, μ is the intercept, \mathbf{x} is an explanatory variable, β the regression
89 coefficient, \mathbf{a} represents the effects due to the phylogenetic structure, \mathbf{b} the effects due to the
90 intraspecific structure, and \mathbf{e} the residuals. \mathbf{x} is a fixed effect (there could be more than one),
91 whereas \mathbf{a} and \mathbf{b} are random effects. The random effects and residuals are assumed to follow
92 normal distributions:

$$\mathbf{a} \sim \mathcal{N}(0, \sigma_a^2 \mathbf{A})$$

$$\mathbf{b} \sim \mathcal{N}(0, \sigma_b^2 \mathbf{B})$$

$$\mathbf{e} \sim \mathcal{N}(0, \sigma_e^2 \mathbf{I}).$$

93 σ_a^2 is the phylogenetic variance, σ_b^2 is the intraspecific variance, and σ_e^2 is the residual variance.
94 The matrices \mathbf{A} and \mathbf{B} represent the phylogenetic and the intraspecific correlation structures,
95 respectively. The identity matrix \mathbf{I} indicates that the residuals are independent and identically

96 distributed. Accordingly, the (co)variance structure (\mathbf{V}) of the model is $\mathbf{V} = \sigma_a^2 \mathbf{A} + \sigma_b^2 \mathbf{B} + \sigma_e^2 \mathbf{I}$.

97 The total variance ($\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_e^2$) can be partitioned in heritable and non heritable por-
98 tions. The heritable portion in the present framework consists of the phylogenetic and intraspecific
99 correlation structures. Hence, the heritable proportion of the total variance is $(\sigma_a^2 + \sigma_b^2)/\sigma^2$. This is
100 akin to the heritability (h^2) parameter in quantitative genetics or the the λ parameter in compar-
101 ative methods (see Housworth et al., 2004, for a discussion). Note that this heritable fraction does
102 not exclusively characterize genetic changes as it can also include non-genetic contributions that
103 can be described by the genetic correlation structures. The remaining variance (σ_e^2), considered
104 non-genetic, includes phenotypic plasticity, measurement error or other effects not defined by the
105 genetic correlation structures.

106 **Phylogenetic generalized least squares**

107 A brief mention of PGLS seems important as it is a popular comparative method. A PGLS model
108 that would include phylogenetic and intraspecific correlation structures could be denoted as:

$$\mathbf{y} \sim \mathcal{N}(\mu + \beta \mathbf{x}, \sigma^2(\delta \mathbf{A} + [1 - \delta] \mathbf{B})). \quad (2)$$

109 In other words, the residuals of the regression are normally distributed according to a correlation
110 structure that is a combination of phylogenetic and intraspecific effects, with respective weights
111 determined by the parameter δ . The main difference with the PMM is the absence of a residual
112 term: in PGLS residuals are completely structured by the genetic correlation matrices provided.
113 This assumption can be relaxed by rescaling the phylogenetic tree to give more or less weight to
114 the terminal branches of the tree (Revell, 2010). We do not consider this PGLS model further here,
115 but it is compared to the PMM using simulations in Appendix S1.

116 **PMM simulations**

117 Simulations were used to examine the performance of the PMM under a suite of conditions where
118 accounting for intraspecific correlations may be important. We simulated data under the PMM
119 model (equation 1) assuming $\mu = 0$ with various relative contributions of the phylogenetic and
120 intraspecific variances, and tested how this affected the estimation of the fixed and random effects.
121 The data simulations followed closely those of Revell (2010) and are described in Appendix S1.
122 One difference is the intraspecific correlation structure that corresponded to the mean variance
123 covariance matrix obtained from 20 independent gene genealogies simulated within a population
124 tree using the Coalescent. The simulations were performed for different regression slopes $\beta \in$
125 $\{0, 0.1, 0.25\}$ and different ratios of intraspecific and interspecific structure ($\sigma_a^2 : \sigma_b^2$) while keeping
126 their sum to 2. In all cases, $\sigma_e^2 = 1$ and $\sigma_x^2 = 2$. Five hundred simulations were performed for
127 each parameter combination. We simulated data with 98 or 100 individuals but different ratios of
128 species vs. individuals per species, specifically 7 : 14, 10 : 10 and 14 : 7. Larger datasets of 20
129 species and 20 individuals per species were also simulated.

130 **Model fitting and performance**

131 The PMM model was fitted using the `MCMCglmm` package in R (Hadfield, 2010). The phylogenetic
132 structure was included in the model by giving the phylogeny to the pedigree argument. The
133 intraspecific structure was incorporated using the genetic intraspecific correlation matrix with a
134 singular value decomposition approach as described in Stone et al. (2011). We used the default
135 priors for the fixed effects and diffuse inverse-Wishart priors for the random effects with $V = 1$
136 and $\nu = 0.002$. We fitted the following models, named according to their respective random
137 effects: 1) *null* (with no genetic structure, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{e}$); 2) *inter* (phylogenetic structure only,
138 $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{e}$); 3) *intra* (intraspecific genetic structure only, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{b} + \mathbf{e}$); 4) *inter+intra*
139 (phylogenetic and intraspecific genetic structures, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{b} + \mathbf{e}$). The MCMC chains
140 were run for 2100 generations, removing the first 100 as burnin and sampling the chain every 10

141 generations. These settings provided good convergence for all models and all simulation parameters.

142 Models were compared for accuracy and precision with regards to the estimation of the fixed
143 effects and for the heritable portion of the variance. Accuracy measures how close the estimated
144 slope is to the true value and precision represents the standard deviation of the estimated slope in
145 each MCMC run. We also report the number of simulations that gave a posterior probability $>$
146 0.95 for the slope to be greater than 0; this estimates the power of the model when $\beta > 0$ and the
147 type I error when $\beta = 0$.

148 **Budburst experiment**

149 We investigated the usefulness of using the PMM on an ecological study design that included
150 both inter- and intraspecific variation. We analyzed a subset of a larger experiment for which we
151 additionally sampled material for genetic analysis. The experiment's objective was to determine
152 the impact of temperature increases and longer photoperiods on the budburst timing for several
153 tree and shrub species (full experiment described in Flynn and Wolkovich, 2018). Clippings from
154 10 species (see Appendix S1) were collected from five individuals at two sites: Harvard Forest (MA,
155 USA; 42.5 °N, 72.2 °W) and the *Station de biologie des Laurentides* (St. Hippolyte, QC, Canada;
156 45.9 °N, 74.0 °W). Clippings were collected in January 2015 and kept cold until the start of the
157 experiment. They were then subjected to different temperatures (15 °C or 20 °C) and photoperiods
158 (8 or 12 hours of light per day) in growth chambers at the Arnold Arboretum. The number of days
159 to budburst was recorded for each clipping.

160 To model the interspecific structure, we pruned a published phylogenetic tree of 32,223 an-
161 giosperm species based on 7 genes (Zanne et al., 2014) and included it in the model as in the sim-
162 ulations. The intraspecific genetic correlation structure was estimated separately for each species
163 from thousands of genome-wide Genotyping-by-Sequencing markers per species (Elshire et al.,
164 2011). Genetic similarities between individuals within species were estimated using `genpofad` (Joly
165 et al., 2015), converted into species variance co-variance matrices, and incorporated in the model
166 with a block diagonal matrix as described for the simulations. Details on our methods and R code

167 are provided in Appendices S1 and S2.

168 The data was analyzed in `MCMCg1mm` with warming, photoperiod, and their interaction as fixed
169 effects and time to budburst as the response variable. For the random effects, we fitted the four
170 models used in the simulations in terms of variance structure. We used the same priors as for
171 the simulated data, but ran the chains for 100,000 generations after a burnin of 5000 generations,
172 sampling every 20 generations. MCMC run convergence was assessed using the potential scale
173 reduction factors (PRSF; converges to 1 with increasing convergence). We used the deviance
174 information criterion (DIC) to compare models. Data and commented scripts to replicate the
175 analyses are presented in Appendix S2.

176 Results

177 Simulations

178 The *null* model without genetic correlation structure always performed worst in terms of precision
179 and accuracy, whereas the *intra* and *inter + intra* models performed best (Fig. 1). The *inter*
180 model with only phylogenetic structure did not perform as well as models *intra* and *inter + intra*,
181 but its performance improved with increasing relative importance of the phylogenetic structure over
182 the intraspecific structure. All models performed better when the intraspecific structure was less
183 important. To estimate the heritable proportion of the total variance, the *inter + intra* model was
184 the most accurate (Fig. 1), although it slightly overestimated the genetic contribution for greater
185 contributions of intraspecific structure. The *inter* model underestimated the genetic structure
186 of the data, but its estimates were greater than the strict interspecific variance included in the
187 simulations. The *intra* model overestimated the total genetic structure of the data even though
188 only the intraspecific structure was modelled.

189 All models had similar type I error rates (Fig. 2), except for the *intra* model that was slightly
190 higher, especially for increasing importance of the phylogenetic structure. The power of the models
191 was similar for $\beta = 0.1$, whereas the models *intra* and *inter + intra* had the best power for $\beta = 0.25$.

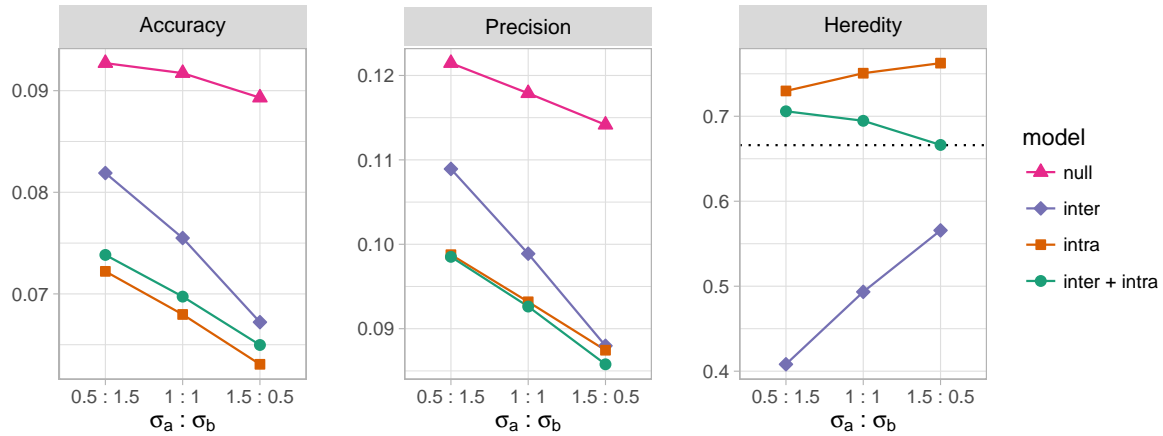


Figure 1: Results of the simulation study for the four variance structure models in terms of slope accuracy and precision, and for estimates of the heritable proportion of the total variance (heredity) with 10 species and 10 individuals per species. Accuracy is the mean absolute distance between the estimated slope ($\hat{\beta}$) and the true slope (β), precision is the mean of the standard deviation of the posterior distribution of $\hat{\beta}$ for each simulation, and heredity is the proportion of the total variance explained by the genetic correlation structure (the dashed line indicates the true value). The x-axis indicates the ratio of phylogenetic (σ_a^2) to intraspecific (σ_b^2) variances used in the simulations. Only the results for $\beta = 0.25$ are shown as these results were not influenced by the slope.

192 The power of model *inter* with $\beta = 0.25$ improved with increasing importance of the phylogenetic
 193 effect and approached the performance of *intra* and *inter + intra* when the phylogenetic was
 194 three times as important as the intraspecific effect (i.e., when $\sigma_a^2:\sigma_b^2 = 1.5 : 0.5$).

195 Varying the amount of population structure had little impact on the results (Appendix S1; Figs.
 196 S1, S2). In contrast, increasing the ratio of the number of species to the number of individuals per
 197 species resulted in an improved relative performance of the *inter* model compared to models that
 198 included the intraspecific structure, but mostly in terms of accuracy (Appendix S1; Figs. S3, S4).
 199 Importantly, the advantage of taking into account intraspecific genetic correlations was also present
 200 when data were simulated for a single species (Figs. S5, S6). Finally, increasing the samples sizes
 201 in the simulations resulted in increased accuracy, precision and power for all methods but did not
 202 affect their relative performances (Figs. S7, S8).

203 Budburst data

204 The number of loci obtained per species ranged from 264 in *Prunus* to 2188 in *Vaccinium* and
 205 was broadly correlated with the genome size of species (see Appendix S1 for detailed information

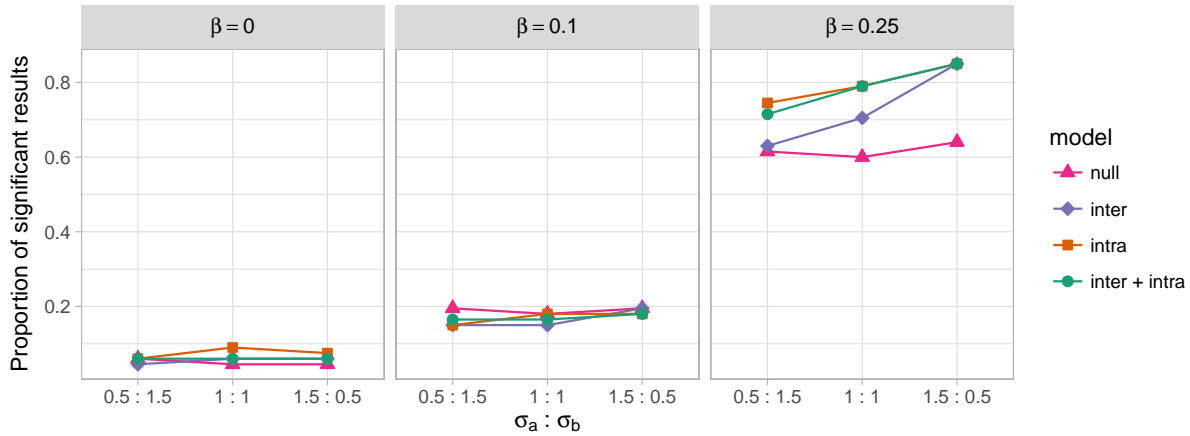


Figure 2: Proportion of the simulations that resulted in a significant regression slope ($\hat{\beta} > 0$) using a threshold of $\alpha = 0.05$. The results for $\beta = 0$ represent the type I error of the models whereas the results with $\beta \in \{0.1, 0.25\}$ represent the power of the models.

206 on the genetic data). The mean locus-based population structure (Φ_{ST}) between sites was similar
 207 across species and ranged from 0.10 to 0.19, suggesting a moderate population structure. Similarly,
 208 phylogenetic trees built from the genetic distances showed that individuals from one site were
 209 generally more similar to individuals from the same site than to individuals from the other site
 210 (Appendix S1).

211 The MCMC runs showed good convergence (PRSF = 1 for fixed and random effects). The
 212 model that best fitted the data was *intra* according to the DIC (2117), followed by *inter + intra*
 213 (2123), *inter* (2135) and *null* (2426). Incorporating intraspecific structure thus resulted in an
 214 important improvement in fit (models *intra* and *inter + intra*), while not accounting for genetic
 215 correlation (*null*) clearly resulted in a poorer fit.

216 The wider posterior intervals obtained for the fixed effects with the *null* model illustrate the
 217 importance of taking into account the genetic structure present in the data (Fig. 3). This was par-
 218 ticularly important for the interaction between warming and photoperiod: the confidence interval
 219 included 0 for the *null* model but not the three other models. The three models that accounted
 220 for the genetic correlation structure gave similar results, but there was a slight improvement in
 221 precision when the intraspecific genetic structure was included. The results suggest that the 5°C
 222 warming treatment had the strongest effect on budburst, followed by a longer (four hours) pho-

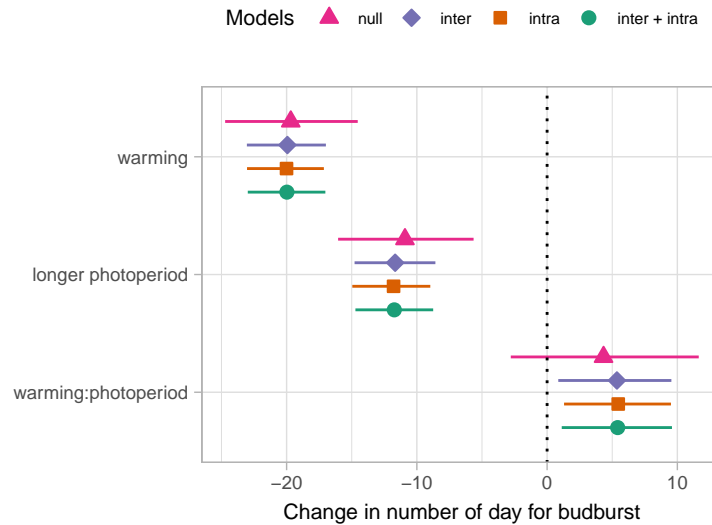


Figure 3: Fixed effects obtained in the phylogenetic mixed model for the four models tested. The symbols represent the means of the posterior distributions and the lines the 95% posterior intervals. The x-axis indicate the change in number of days for budburst, with negative number indicating that buds are opening earlier.

Table 1: Mean proportion of the total variance explained by the random effects of the models fitted to explain change in days for budburst, with their 95% posterior intervals (in brackets). The heredity (h^2) and the proportion of the total genetic structure due to the intraspecific correlation ($\sigma_b^2/(\sigma_a^2 + \sigma_b^2)$) are given for models where they can be estimated.

Models	σ_a^2	σ_b^2	σ_e^2	h^2	$\sigma_b^2/(\sigma_a^2 + \sigma_b^2)$
<i>null</i>	–	–	1 [1,1]	–	–
<i>inter</i>	0.65 [0.44, 0.85]	–	0.35 [0.15,0.56]	0.65 [0.44, 0.85]	–
<i>intra</i>	–	0.68 [0.30, 0.94]	0.32 [0.06,0.70]	0.68 [0.30, 0.94]	–
<i>inter + intra</i>	0.21 [4×10^{-6} , 0.79]	0.47 [1×10^{-5} , 0.93]	0.31 [0.05,0.66]	0.69 [0.34,0.95]	0.69 [2×10^{-5} ,0.99]

223 toperiod (Fig. 3). The interaction between these effects was positive and significant (except for the
 224 *null* model), suggesting that they are not additive.

225 Regarding the partitioning of the variance, the genetic correlation structures explained about
 226 two-thirds of the total variance for all models (Table 1). The *inter + intra* model that partition the
 227 genetic variance into phylogenetic and intraspecific further suggests that the intraspecific variance
 228 is slightly greater than the interspecific variance, but the confidence interval was huge suggesting
 229 these variances components are difficult to estimate with precision in this dataset (Table 1).

230 Discussion

231 Accounting for the intraspecific genetic correlation structure

232 An increasing number of studies mention the potential importance of accounting for intraspecific
233 genetic structure in multi-species studies. Our results from simulations and empirical data showed
234 multiple advantages of this approach. Perhaps most importantly, incorporating intraspecific cor-
235 relation structure in statistical models led to a gain in accuracy and precision of the fixed effects,
236 which are generally the parameters of interest in a study. Both the simulations and the empirical
237 studies highlighted this result. The simulations further showed that this advantage persisted under
238 various conditions including when there were no phylogenetic effects (single-species analyses).

239 The models incorporating intraspecific genetic correlations performed well because they parti-
240 tion the total variance in the data—that would otherwise be classified mostly to the error term—to
241 the genetic correlation structure(s). This is shown by the higher values of the variance due to
242 heritable effects in models that included intraspecific genetic correlations (Fig. 1; Table 1).

243 In addition to providing more accurate fixed effects, the finer partitioning of the total variance
244 gives a better understanding of the study system by quantifying the proportion of the variance that
245 is due to the intraspecific genetic structure. Notably, the improved performance observed when
246 incorporating the intraspecific structure is not due to the specific modelling framework used in this
247 study, namely the phylogenetic mixed model. Indeed, the simulations we performed under a PGLS
248 model that incorporates intraspecific structure also showed a marked improvement in performance
249 compared to standard ordinary least squares (Appendix S1; Figs. S7, S8).

250 One surprising result was the very good performance of the model that included only the in-
251 traspecific correlation structure (*intra*), which performed nearly as well as the model that accounted
252 for both phylogenetic and the intraspecific genetic structures (*inter + intra*) in terms of accuracy
253 and precision in simulations. This result may lead some researchers to consider including only the
254 intraspecific structure, but we advise against it. First, the relative performance of the *intra* model
255 decreased relative to the *inter + intra* model when the importance of the intraspecific correlation

256 structure decreased relative to the phylogenetic variance (Fig. 1). Second, the *inter + intra* model
257 provided more precise estimates of the proportion of the total variance that is genetically struc-
258 tured in our simulations (Fig. 1) and more precise estimates of fixed effects across a wide range
259 of parameters. And last, as our fundamental biological understanding of ecological questions often
260 stresses the multilevel nature of individuals within species, we argue it is important to include both
261 structures in analyses.

262 **The Phylogenetic Mixed Model**

263 The phylogenetic mixed model (PMM) offers more flexibility than other comparative methods
264 (Hadfield and Nakagawa, 2010). One advantage is that it uses a terminology familiar to most
265 ecologists. The phylogenetic and the intraspecific genetic correlation structures are considered
266 “random effects” in the model, similar to how blocks are often treated in a randomized block
267 design. That is, the model assumes that they add variance to the species response in a structured
268 way that can be estimated and removed from the residual error, resulting in improved performance
269 of the model. Further, because the residual variance is estimated by the model (in contrast to other
270 methods, see Hadfield and Nakagawa, 2010), model performance is not affected if the intraspecific
271 correlation structure has little effect on the data; in such cases the estimated variance due to
272 intraspecific structure will simply be small.

273 Another advantage of the PMM is that it allows modeling several random effects simultaneously
274 (Garamszegi, 2014). In our analyses, the total variance of the model included a phylogenetic
275 fraction, an intraspecific fraction, and a residual fraction. But it would be straight-forward to also
276 add a random effect that could account for measurement error, given the appropriate study design.
277 In contrast, the PGLS approach we introduced only considered a phylogenetic and an intraspecific
278 variance with no residual error, which likely explains the increased Type I error compared to the
279 PMM as residual errors were included in the simulations (Appendix S1).

280 Finally, the PMM is particularly well suited for experimental studies that include several fixed
281 effects as in the present study. In this regard, this study differs from many comparative studies

282 in that the samples studied were subject to experimental treatments. In such experiments, each
283 species has several values for the response variable; at least one per fixed effect. Although such
284 datasets can be analysed using most comparative methods, which often necessitate duplicating
285 the terminal branches of the phylogeny to have—for each species—one tree tip that matches each
286 observation for the response variable, the analysis is much more intuitive with PMM. For instance,
287 the phylogenetic correlation structure can easily be included in the model by associating the species
288 on the phylogeny to the factor representing the species in the dataset (see Appendix S2).

289 **Modelling guidelines**

290 The importance of accounting for intraspecific genomic relatedness will depend on the importance
291 of the intraspecific genetic structure. Our results showed that the advantages gained from this
292 approach are more important with greater population structure (obtained with smaller effective
293 population sizes, restricted gene flow and longer divergence times) and when the intraspecific vari-
294 ance has a greater relative importance compared to the phylogenetic variance (provided that the
295 phylogenetic structure is corrected for).

296 The gain from modelling intraspecific correlation structure also depends on the genetic bases of
297 the traits studied and the relevance of the explanatory variable(s) used. In our example, budburst
298 is known to have strong responses to the environmental cues used in the experiment (warming and
299 photoperiod), suggesting these are important explanatory variables. Yet, budburst has also recently
300 been found to be particularly plastic across populations (Aitken and Bemmels, 2016). It is thus
301 possible that the study of other traits with a stronger genetic basis (e.g., timing of budset) could
302 have resulted in larger improvements when accounting for the intraspecific correlation structure.

303 On a practical aspect, we used the terminology inter- and intraspecific in this study, but the
304 delimitation between the two genetic correlations structures does not have to be at the species
305 level. The decision should be taken depending on the nature of the study. In some cases, it might
306 be logical to have a genetic structure above and below subspecies, and in others such as in recent
307 species complexes it might be interesting to characterize the genetic correlations between closely

308 related species using genome wide markers to capture the complex mosaic structure of genomes.

309 Comparative methods are being increasingly used to correct for the phylogenetic non-independence
310 of species in statistical tests, in part because of the ease with which one can obtain a well resolved
311 phylogeny. Our results show that important gains can also be obtained by accounting for the
312 intraspecific genetic structure.

313 **Data and supplementary information**

314 Additional tables and figures are available in Appendix S1 and commented R scripts in Appendix S2.
315 Raw Genotyping-by-sequencing reads have been deposited in the National Center for Biotechnology
316 Information (NCBI) Sequence Read Archive (SRA) under accession number SRP126957. Further
317 scripts, budburst data and processed sequence data will be deposited in a public archive.

318 **Competing interests**

319 We have no competing interests.

320 **Author's contribution**

321 SJ and EW conceived the work, DFBF and EW collected the data, SJ analysed the data, SJ and
322 EW wrote the manuscript. All authors gave final approval for publication.

323 **Acknowledgements**

324 The authors would like to thank T. Savas, J. Samaha, and H. Eyster for help with field collections
325 of tissue for genetic analyses, and constructive comments by Jonathan Davies, Joe Felsenstein, and
326 an anonymous reviewer.

327 **Funding**

328 This work was financially supported by the William F. Milton Fund (Harvard University).

329 References

- 330 Aitken, S. N. and J. B. Bemmels, 2016. Time to get moving: assisted gene flow of forest trees. *Evol*
331 *Appl* 9:271–290.
- 332 Alofs, K. M., 2016. The influence of variability in species trait data on community-level ecological
333 prediction and inference. *Ecol and Evol* 6:6345–6353.
- 334 Anderson, B. J., H. R. Akcakaya, M. B. Araujo, D. A. Fordham, E. Martinez-Meyer, W. Thuiller,
335 and B. W. Brook, 2009. Dynamics of range margins for metapopulations under climate change.
336 *P Roy Soc Lond B Bio* 276:1415–1420.
- 337 Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon, 2008.
338 Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*
339 320:800–803.
- 340 Chen, I. C., J. K. Hill, R. Ohlemuller, D. B. Roy, and C. D. Thomas, 2011. Rapid range shifts of
341 species associated with high levels of climate warming. *Science* 333:1024–1026.
- 342 Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler, and S. E.
343 Mitchell, 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity
344 species. *PLoS ONE* 6:e19379.
- 345 Felsenstein, J., 1985. Phylogenies and the comparative method. *Am Nat* 125:1–15.
- 346 ———, 2002. Contrasts for a within-species comparative method. Pp. 118–129, *in* M. Slatkin and
347 M. Veuille, eds. *Modern developments in theoretical population genetics: the legacy of Gustave*
348 *Malécot*. Oxford Univeristy Press, Oxford, UK.
- 349 ———, 2008. Comparative methods with sampling error and within-species variation: contrasts
350 revisited and revised. *Am Nat* 171:713–725.
- 351 Flynn, D. F. B. and E. M. Wolkovich, 2018. Temperature and photoperiod drive spring phenology
352 across all species in a temperate forest community. *New Phytologist* 219:1353–1362.

- 353 Funk, J. L., J. E. Larson, G. M. Ames, B. J. Butterfield, J. Cavender-Bares, J. Firn, D. C. Laughlin,
354 A. E. Sutton-Grier, L. Williams, and J. Wright, 2017. Revisiting the holy grail: using plant
355 functional traits to understand ecological processes. *Biol Rev* 92:1156–1173.
- 356 Garamszegi, L. Z., 2014. Uncertainties due to within-species variation in comparative studies:
357 measurement errors and statistical weights, book section 7, Pp. 157–199. Springer, Berlin.
- 358 Gienapp, P., S. Fior, F. Guillaume, J. R. Lasky, V. L. Sork, and K. Csilléry, 2017. Genomic
359 quantitative genetics to study evolution in the wild. *Trends in Ecology & Evolution* 32:897–908.
- 360 Grafen, A., 1989. The phylogenetic regression. *Philos T Roy Soc B* 326:119–157.
- 361 Hadfield, J. D., 2010. MCMC methods for multi-response generalized linear mixed models: The
362 MCMCglmm R package. *J Stat Softw* 33:1–22.
- 363 Hadfield, J. D. and S. Nakagawa, 2010. General quantitative genetic methods for comparative biol-
364 ogy: phylogenies, taxonomies and multi-trait models for continuous and categorical characters.
365 *J Evolution Biol* 23:494–508.
- 366 Hansen, T. F., W. S. Armbruster, and L. Antonson, 2000. Comparative analysis of character
367 displacement and spatial adaptations as illustrated by the evolution of *Dalechampia* blossoms.
368 *The American Naturalist* 156:S17–S34.
- 369 Housworth, E. A., E. P. Martins, and M. Lynch, 2004. The phylogenetic mixed model. *Am Nat*
370 163:84–96.
- 371 Ives, A. R., P. E. Midford, and T. Garland, 2007. Within-species variation and measurement error
372 in phylogenetic comparative methods. *Syst Biol* 56:252–270.
- 373 Joly, S., D. Bryant, and P. J. Lockhart, 2015. Flexible methods for estimating genetic distances
374 from single nucleotide polymorphisms. *Methods Ecol Evol* 6:938–948.
- 375 Lynch, M., 1991. Methods for the analysis of comparative data in evolutionary biology. *Evolution*
376 45:1065–1080.

- 377 Read, Q. D., S. M. Hoban, M. B. Eppinga, J. A. Schweitzer, and J. K. Bailey, 2016. Accounting for
378 the nested nature of genetic variation across levels of organization improves our understanding
379 of biodiversity and community ecology. *Oikos* 125:895–904.
- 380 Revell, L. J., 2010. Phylogenetic signal and linear regression on species data. *Methods Ecol Evol*
381 1:319–329.
- 382 Stone, G. N., S. Nee, and J. Felsenstein, 2011. Controlling for non-independence in comparative
383 analysis of patterns across populations within species. *Philos T Roy Soc B* 366:1410–1424.
- 384 Villemereuil, P. d. and S. Nakagawa, 2014. General quantitative genetic methods for comparative
385 biology. Pp. 287–303, *in* L. Z. Garamszegi, ed. *Modern phylogenetic comparative methods and*
386 *their application in evolutionary biology*. Springer-Verlag, Berlin, Heidelberg.
- 387 Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier,
388 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecol*
389 *Evol* 27:244–252.
- 390 Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis, 2008. Phylogenetic
391 patterns of species loss in Thoreau’s woods are driven by climate change. *P Natl Acad Sci USA*
392 105:17029–17033.
- 393 Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, R. G. FitzJohn, D. J.
394 McGlinn, B. C. O’Meara, A. T. Moles, P. B. Reich, D. L. Royer, D. E. Soltis, P. F. Stevens,
395 M. Westoby, I. J. Wright, L. Aarssen, R. I. Bertin, A. Calaminus, R. Govaerts, F. Hemmings,
396 M. R. Leishman, J. Oleksyn, P. S. Soltis, N. G. Swenson, L. Warman, and J. M. Beaulieu, 2014.
397 Three keys to the radiation of angiosperms into freezing environments. *Nature* 506:89–92.